3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

# Product Information

**iPSC β-ACTIN RFP**
**Induced Pluripotent Stem Cells**

Catalog Number **IPSC1028**
Storage Temperature –196 °C, liquid nitrogen

## TECHNICAL BULLETIN

### Product Description

Induced pluripotent stem cells (iPS cells or iPSCs) are derived from adult somatic cells by induction of expression of specific genes.[1-2] Similar to embryonic stem cells, iPSCs are capable of differentiating into multiple cell lineages representing all three germ layers, and can form embryoid bodies and teratomas.

iPSC β-ACTIN RFP cells are produced by OSKM retrovirus reprogramming of epithelial cells from a Caucasian female 24 years of age with no known genetic disorders. iPSC β-ACTIN RFP cells have *ACTB* regulated expression of RFP. Six different isoforms of actin are known in humans. β-Actin is one of two non-muscle cytoskeletal actins involved in cell motility, structure, and integrity.

Cell Line Description
iPS cells
Source Tissue: Epithelium
Gender: Female
Age: 24
Ethnicity: Caucasian
Reprogramming method used: OSKM retrovirus

### Component

| | |
|---|---|
| iPSC β-ACTIN RFP<br>(human iPSCs, >1.0 × $10^6$ cells)<br>Catalog Number IPSC1028<br>Frozen as a suspension of single cells in CryoStor® CS10 cell cryopreservation medium (Catalog Number C2874) | 1 vial |

### Reagents and Equipment Required but Not Provided.

Products for Feeder-Free Stem Cell Culture System
Stemline® Pluripotent Stem Cell Culture Medium (Catalog Number S1202)
ECM Gel from Engelbreth-Holm-Swarm murine sarcoma (Catalog Number E1270)
DMEM/F12 medium (Catalog Number D6421)
Hanks' Balanced Salt solution (HBSS, Catalog Number H6648)
Y-27632 dihydrochloride (Catalog Number Y0503)
Accutase® solution (Catalog Number A6964)
CryoStor® CS10 cell cryopreservation medium (Catalog Number C2874)
Corning® cell lifter (Catalog Number CLS3008)

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Biosafety Level: 1
This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. Appropriate safety procedures are recommended to be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures have been published.[3-6]

### Preparation Instructions

10 mM Y-27632 Stock Solution (1,000×) – Dissolve Y-27632 dihydrochloride (Catalog Number Y0503) in DMSO (1 mg/0.3 mL) and vortex thoroughly.

Stemline Complete Medium –

Stemline Pluripotent Stem Cell Culture Medium is supplied as two components, which are combined prior to use.

1. Thaw the basal medium (Catalog Number S0952) overnight at 2–8 °C.
2. Thaw the supplement (Catalog Number S1077) at room temperature before addition to the basal medium. Centrifuge the Supplement tube.
3. Under sterile conditions, transfer the contents of the supplement tube into the bottle of basal medium.
4. Gently swirl the bottle to mix.

Note: **Do not refreeze the Stemline Complete Medium.** The Stemline Complete Medium should be stored at 2–8 °C and used within 14 days.

Accutase Solution with 10 μM Y-27632 –

1. Thaw Accutase solution (Catalog Number A6964) overnight at 2–8 °C.
2. Add 1 μL of 10 mM Y-27632 Stock Solution (1,000×) per 1 mL of Accutase solution (final Y-27632 concentration is 10 μM).
3. Store working aliquots at –20 °C.

Coating culture vessels with ECM Gel –

ECM Gel (Catalog Number E1270) is recommended for use with Stemline Complete Medium.

Note: The ECM Gel will gel within 5 minutes at 20 °C. For prolonged manipulations, it should be maintained below 10 °C under strict aseptic conditions.

1. Thaw the ECM Gel overnight on ice or at 2–8 °C prior to use.
2. Dilute the ECM Gel 100-fold with ice-cold DMEM/F12 medium (Catalog Number D6421) and mix well.
3. Coat each cell culture dish with an appropriate volume of diluted ECM gel to cover the entire surface.
4. Swirl the culture dish to ensure the entire area is coated sufficiently.
5. Incubate at 37 °C for one hour prior to use.
6. Aspirate the ECM and rinse with phosphate buffered saline.
7. Add warm Stemline Complete Medium with 10 μM Y-27632 to the dish.

Unused ECM coated plates may be stored at 37 °C in serum free medium for up to 7 days.

**Storage/Stability**

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase. Extended storage at –70 °C will result in loss of viability.

**Procedures**

Please take time to carefully read through these procedures to ensure optimal performance. Human iPSC β-ACTIN RFP cells expand optimally in Stemline Pluripotent Stem Cell Culture Medium. Contact Sigma-Aldrich Technical support for additional guidance: techserv@sial.com

Thawing iPSC β-ACTIN RFP Cells into Stemline Complete Medium

Note: Thaw into Stemline Complete Medium supplemented with 10 μM Y-27632 to improve cell survival.

1. Prepare Stemline Complete Medium with 10 μM Y-27632 – Add 11 μL of 10 mM Y-27632 Stock Solution (1,000×) to 11 mL of Stemline Complete Medium.
2. Allow culture reagents to come to room temperature prior to use (less than 30 minutes). Shield culture reagents from light.
3. Thaw the cryovial containing the iPSC β-ACTIN RFP cells by gentle agitation in a 37 °C water bath.
4. Remove the vial from the water bath as soon as the contents are thawed and spray with 70% ethanol. All operations from this point forward should be carried out aseptically.
5. Using a 1 mL pipette, transfer the entire contents of the vial into a 15 mL conical tube.
6. Slowly add 9 mL of Stemline Complete Medium with 10 μM Y-27632 to the 15 mL conical tube.
7. Rinse the vial with 1 mL of Stemline Complete Medium with 10 μM Y-27632 and add this to the 15 mL conical tube.
8. Centrifuge the cells for 5 minutes at 200 × *g*.
9. Aspirate the medium and resuspend the cells in 1 mL of Stemline Complete Medium with 10 μM Y-27632.
10. Seed cells to ECM-coated cell culture dishes at a density of ~20,000 cells/cm$^2$ in Stemline Complete Medium with 10 μM Y-27632. Incubate at 37 °C in a 5% $CO_2$ atmosphere.
11. Change medium daily, see Procedure, Feeding.

Feeding iPSC β-ACTIN RFP Cells – Medium Change Frequency

Daily replacement of the culture medium is recommended. A volume of ~0.2 mL of Stemline Complete Medium **without** Y-27632 should be used for each square centimeter ($cm^2$) of culture surface.

Passaging

Note: Human iPSC β-ACTIN RFP cells typically need to be passaged every 3–4 days. If the culture is allowed to overgrow, the iPS cells will begin to differentiate. The cells should be passaged when the culture reaches ~80% confluence. When cultured with Stemline Pluripotent Stem Cell Medium, iPSC β-ACTIN RFP cells may be passaged by enzymatic (using Accutase) or mechanical dissociation.

1. Enzymatic Dissociation
   Note: For enzymatic dissociation of iPSC β-ACTIN RFP cells, use the Accutase Solution with 10 μM Y-27632 and Stemline Pluripotent Stem Cell Culture Medium with 10 μM Y-27632 to ensure cell survival.
   a. Under aseptic conditions, remove culture medium from cells, rinse with pre-warmed HBSS (Catalog Number H6648), and aspirate buffer.
   b. Dissociate cells using pre-warmed Accutase Solution with 10 μM Y-27632 for 3–5 minutes at 37 °C.
   c. Add an equal volume of Stemline Pluripotent Stem Cell Culture Medium with 10 μM Y-27632.
   d. If the cells are not fully detached from the cell culture surface, gently lift them off using a cell lifter (Catalog Number CLS3008).
   e. Transfer the entire contents to a 15 mL conical tube and centrifuge for 5 minutes at 200 × *g*.
   f. Aspirate the medium and resuspend the cells in 1 mL of Stemline Pluripotent Stem Cell Culture Medium with 10 μM Y-27632.
   g. Seed cells to an ECM-coated cell culture dish at a density of ~20,000 cells/$cm^2$ with Stemline Pluripotent Stem Cell Culture Medium with 10 μM Y-27632.
   h. Change medium daily, see Procedure, Feeding.
2. Mechanical Dissociation
   a. Under aseptic conditions, remove culture medium from the cells aseptically, rinse with pre-warmed HBSS, and aspirate buffer.
   b. Add fresh Stemline Pluripotent Stem Cell Culture Medium with 10 μM Y-27632.
   c. Using a sterile pipette tip or a flame drawn glass pipette, gently score the colonies to break into clusters of a few hundred cells.
   d. Gently lift cells off using a cell lifter (Catalog Number CLS3008).
   e. Gently pipette the cells up and down several times to mix thoroughly and break up larger clumps.
   f. Seed cells to an ECM-coated cell culture dish at the desired cell density in Stemline Complete Medium with 10 μM Y-27632.
   g. Change medium daily, see Procedure, Feeding.

Cell cryopreservation

After enzymatic or mechanical dissociation, cells can be cryopreserved.

1. Dissociate cells as described in Procedure, Passaging.
2. Transfer the cells to a 15 mL conical tube and centrifuge for 5 minutes at 200 × *g*.
3. Aspirate the medium and resuspend the cells in cold CryoStor cell cryopreservation medium (Catalog Number C2874) with 10 μM Y-27632 at $1 \times 10^6$ cells/mL.
4. Transfer the cells to a cryovial and place in an appropriate freezing container at –80 °C. For longer term storage, it is recommended to store the cells in liquid nitrogen.

**References**


1. Takahashi, K. et al. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell, **131**, 1-12 (2007).
2. Yu, J. et al. Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells. Science, **318**, 1917-1920 (2007).
3. Fleming, D.O. et al., (1995) Laboratory Safety: Principles and Practice. 2nd edition, ASM press, Washington, DC.
4. Hay, R.J. et al., eds. (1992) ATCC Quality Control Methods for Cell Lines, 2nd edition, Publishes by ATCC.
5. Caputo, J.L., Biosafety procedures in cell culture. J. Tissue Culture Methods, **11**, 223-227 (1998).
6. Centers for Disease Control (1999), Biosafety in Microbiological and Biomedical Laboratories Human Health Service Publication No. (CDC) 93-8395. U.S. Dept of Health and Human Services; 4th Edition U.S. Government Printing Office, Washington, D.C. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm


Label Licenses

1. Sigma-Aldrich (SA) Licensed Pluripotent Products - [Licensed iPS Cells and Licensed iPS Cell Kit Products (components used to produce iPS Cells)]

   (i) End User shall not use SA Pluripotent Products and its derivatives for uses other than for End User's internal research use; End User may not use the SA Pluripotent Products and its derivatives for Commercial Purpose*.
   (ii) If End User wishes to use SA Pluripotent Products and its derivatives for Commercial Purpose, End User shall contact IPS Academia Japan, Inc. to negotiate a requisite license.
   (iii) End User shall not transfer, sell or supply any SA Pluripotent Products and its derivatives to Third Parties, except that End User may transfer SA Pluripotent Products and its derivatives solely to bona-fide collaborators and/or sub-contractors who perform research activities excluding Commercial Purpose*solely for End User's research (but not for End User's financial gain) on behalf of and under the direct control of the End-User.
   (iv) End User shall not use SA Pluripotent Products and its derivatives for administration and use for humans/animal therapeutic, diagnostic and/or prophylactic purposes including but not limited to clinical applications, cell therapy, transplantation, and/or regenerative medicine.
   (v) No other right, express or implied is conveyed by the sale of SA Pluripotent Products.

   *Commercial Purpose includes use of the SA Pluripotent Products and its derivatives, (i) for the manufacture of related products (i.e., culture medium and equipment) distributed or sold to a Third Party; (ii) to provide a service, information or data to a Third Party; and (iii) for screening commercially active compounds for the purpose of developing the compound for commercial sale.

2. Licensed Differentiated Cells

   (i) End User shall not use Licensed Differentiated Cells for uses other than for End User's internal research use. For clarity, End User is allowed to use Licensed Differentiated Cells for research use in screening applications, including high-throughput screens inclusive of small molecules, antibodies, proteins, peptides, miRNAs, and large molecule screening.
   (ii) End User may transfer, distribute or supply Licensed Differentiated Cells to Third Parties in accordance with section 2(iii) of the Customer Notice.
   (iii) End User shall not use Licensed Differentiated Cells for administration and use for humans/animal therapeutic, diagnostic and/or prophylactic purposes including but not limited to clinical applications, cell therapy, transplantation, and/or regenerative medicine.
   (iv) No other right, express or implied is conveyed by the sale of Licensed Differentiated Cells

This product is for research field use only. For information on commercial licensing, contact Licensing Department, Evrogen, email: license@evrogen.com

This Product and its use are the subject of one or more of the following patents controlled by Sangamo BioSciences, Inc.: U.S. Patent Nos. 6,534,261, 6,607,882, 6,746,838, 6,794,136, 6,824,978, 6,866,997, 6,933,113, 6,979,539, 7,013,219, 7,030,215, 7,220,719, 7,241,573, 7,241,574, 7,585,849, 7,595,376, 6,903,185, 6,479,626, US20030232410, US20090203140 and corresponding foreign patent applications and patents. BEFORE OPENING OR USING THIS PRODUCT, PLEASE READ THE TERMS AND CONDITIONS SET FORTH IN THIS LICENSE AGREEMENT. YOUR USE OF THIS PRODUCT SHALL CONSTITUTE ACKNOWLEDGMENT AND ACCEPTANCE OF THESE TERMS AND CONDITIONS.

If you do not agree to use this Product pursuant to the terms and conditions set out in this License Agreement, please contact Sigma Technical Services within ten days of receipt to return the unused and unopened Product for a full refund; provided, however, that custom-made Products may not be returned for a refund.

The purchase of this Product conveys to you, the buyer, the non-transferable right to use the purchased Product for Licensed Research Use (see definition below) subject to the conditions set out in this License Agreement. If you wish to use this Product for any purpose other than Licensed Research Use, you must first obtain an appropriate license (see information set out below).

This Product may not be used for any purpose other than Licensed Research Use. Your right to use this Product for Licensed Research Use is subject to the following conditions and restrictions:

1. "Licensed Research Use" means any use for research purposes, other than:

(a) Licensing, selling, distributing, or otherwise providing Modified Animals to any third party other than Sigma and its affiliates as provided herein: provided however, that you may provide Modified Animals to researchers within your research organization located at the same research facility or campus. A "Modified Animal" means an animal having a genomic modification at the target site that results from Customer's use of the Product. Modified Animal includes but is not limited to (a) heterozygotes and mosaic animals, (b) the descendants of Modified Animals, (c) animals created from the breeding of Modified Animals with other animals, and (d) animals created by the Customer which contain and/or incorporate genetic information derived from Modified Animals.

(b) GMP production of therapeutic, diagnostic, prophylactic or other medicinal Products intended for use in humans or non-human animals, or any other industrial use solely to the extent involving commercial sale of a Product or service. If a molecule or any derivative of such molecule is used in or administered to humans, then the production of such molecule shall be deemed to be GMP production and therefore in violation of this License Agreement;

(c) use for gene targeting and/or gene regulation to modify the genome of a plant cell, plant, or plant cell culture (in each case, whether constituting or derived from a vascular or non-vascular plant), or alter the nucleic acid or protein expression in a plant cell, plant, or plant cell culture. "Non-vascular" plants shall include but not be limited to algae, moss, and fungi; and

(d) modification or reverse-engineering of the Product in any way or creating any derivatives or sequence variants thereof.

2. You may not transfer the Product, its components, or any materials made through the use of this Product, including Modified Animals, to any third party without prior written approval of Sigma and without the transferee entering into a material transfer agreement with Sigma. Notwithstanding the foregoing:

(a) the Product or materials made through use of the Product may be transferred by you to your legal affiliates or bonafide third party contractors performing paid work on your behalf, with the exception of creation of Modified Animals, provided the use by such third party contractors is limited to performance of work for you; and

(b) you may donate Mice that are Modified Animals as defined above ("Modified Mice") to The Jackson Laboratory, a licensed distributor of Modified Mice.

3. You may not transfer the Product or materials made through use of the Product to third party contractors performing paid work on your behalf for the purposes of creation of Modified Animals.

4. Your right to use the Product will terminate immediately if you fail to comply with these terms and conditions. You shall, upon such termination of your rights, destroy all Product, Modified Animals, and components thereof in your control, and notify Sigma of such in writing.

5. You may not use the Product to support the filing of a patent application in any country in the world that contains claims directed to the Product or its uses.

For information on purchasing a license to this Product for purposes other than Licensed Research Use, contact your local Sigma Sales representative, who will refer you to the proper licensing representative, or in the USA call 800-325-3010.

For information on donating Modified Mice to The Jackson Laboratory, please visit their website at: http://www.jax.org/grc/index.html.

Stemline is a registered trademark of Sigma-Aldrich Co. LLC.

Accutase is a registered trademark of Innovative Cell Technologies Inc

CryoStor is a registered trademark of BioLife Solutions, Inc

Corning is a registered trademark of Corning, Inc.

JC,MAM 04/13-1